Assessment of Ex-Vitro Anaerobic Digestion Kinetics of Crop Residues Through First Order Exponential Models: Effect of LAG Phase Period and Curve Factor

ABDUL RAZAQUE SAHITO*, RASOOL BUX MAHAR**, AND KHAN MUHAMMAD BROHI**

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ABSTRACT

Kinetic studies of AD (Anaerobic Digestion) process are useful to predict the performance of digesters and design appropriate digesters and also helpful in understanding inhibitory mechanisms of biodegradation. The aim of this study was to assess the anaerobic kinetics of crop residues digestion with buffalo dung. Seven crop residues namely, bagasse, banana plant waste, canola straw, cotton stalks, rice straw, sugarcane trash and wheat straw were selected from the field and were analyzed on MC (Moisture Contents), TS (Total Solids) and VS (Volatile Solids) with standard methods. In present study, three first order exponential models namely exponential model, exponential lag phase model and exponential curve factor model were used to assess the kinetics of the AD process of crop residues and the effect of lag phase and curve factor was analyzed based on statistical hypothesis testing and on information theory. Assessment of kinetics of the AD of crop residues and buffalo dung follows the first order kinetics. Out of the three models, the simple exponential model was the poorest model, while the first order exponential curve factor model is the best fit model. In addition to statistical hypothesis testing, the exponential curve factor model has least value of AIC (Akaike's Information Criterion) and can generate methane production data more accurately. Furthermore, there is an inverse linear relationship between the lag phase period and the curve factor.

Key Words: **First Order Kinetics, Anaerobic Digestion, Lag Phase Period, Curve Factor.**

1. INTRODUCTION

Anaerobic digestion is a biological process in which a group of microorganisms biodegrades the organic matter (substrate) in the absence of free molecular oxygen. As a result of this complex biological process, organic matter is mainly converted into a mixture of methane and carbon dioxide [1]. Methane can be produced from a wide range of crops, animal manures and other organic wastes including organic fraction of municipal solid waste, agro-industrial wastes and crop residues [2-4], and thus it offers high flexibility and can be adapted to the specific needs of different locations and farm management [5].

In the agricultural sector, one possible solution to process crop residues is co-digestion with animal dungs, which is the largest agricultural waste stream. In addition to the

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* Assistant Professor, Mechanical Engineering Department, Mehran University of Engineering & Technology Jamshoro.
** Professor, Institute of Environmental Engineering & Management Mehran University of Engineering & Technology Jamshoro.

production of renewable energy, controlled AD of animal manures reduces emissions of greenhouse gases, nitrogen and odor from dung management, and intensifies the recycling of nutrients within agriculture [6]. Kinetic studies of AD process are useful to predict the performance of digesters and design appropriate size of digesters and are also helpful in understanding inhibitory mechanisms of biodegradation [7]. It is difficult to model digestion process because many different groups of bacteria are involved; especially methanogenic bacteria have a much lower growth rate than the acid producing ones. As the conversion of acids to biogas is generally considered to be a rate limiting step of the overall reaction, the methanogenic phase is usually used for modeling purposes [8].

The purpose of present study is to assess the anaerobic kinetics of the digestion of crop residues with buffalo dung. The assessment of the kinetics was carried out by using first order exponential model, exponential lag phase model and exponential curve factor model and the effect of lag phase and curve factor was analyzed based on statistical hypothesis and on information theory.

2. METHODOLOGY

2.1. Crop Residues and Inoculum

Seven crop residues i.e. sugarcane bagasse, sugarcane trash, banana plant waste, canola straw, cotton stalks, rice straw and of wheat straw were selected as the substrate for the anaerobic co-digestion. These crop residues were dried at atmospheric temperature and moisture content was determined, and then shredded with the axial bar hammer mill followed by the coffee grinder. The size of the crop residue samples was less than 10mm and the quantity of shredded residue was 10-15 kg. In order to obtain the representative samples of the crop residues, the coining and quartering method was used and about one kg shredded crop residue sample was taken for grinding purpose. The final size of the crop residues was less than or equals to one millimeter.

The inoculum utilized in the present study was the admixture of fresh buffalo dung and digestate. The fresh buffalo dung was acquired from the small animal farmhouse located near the Mehran University of Engineering & Technology, Jamshoro, Pakistan and digestate was acquired from the laboratory scale continuously stirred tank anaerobic digester reactor operating at mesophilic temperature of 37±0.2°C. On the basis of mass of volatile solids contained by the buffalo dung and digestate, the admixture ratio was set to 9:1 for buffalo dung and digestate respectively.

The analysis of crop residues and the inoculum were carried out by using gravimetric method and MC (Moisture Content), TS (Total Solids) and VS (Volatile Solids) were determined as per APHA, Standard Methods [9]. The results of the analyses are given in Table 1.

2.2 The BMP Test

The BMP (Biochemical Methane Potential) test was carried out through AMPTS. This automatic system follow similar standard as the formal methane potential tests. Though, in the AMPTS the measurement of cumulative methane production and methane flow rate from the anaerobic digestion of organic material is recorded automatically during the period of incubation. Furthermore, the AMPTS also compensate the methane gas at STP (Standard

TABLE 1. ANALYSIS OF CROP RESIDUES AND INOCULUM

Parameter	Bagasse	Banana Plant Waste	Canola Straw	Cotton Stalks	Rice Straw	Sugarcane Trash	Wheat Straw	Inoculum
MC (%)	6.13	85.53	6.56	6.61	2.12	2.36	7.14	82.30
TS (%)	93.87	14.47	93.44	93.39	97.88	97.64	92.86	17.70
VS (%)	92.26	12.01	84.85	89.34	81.58	84.99	80.58	12.44

Temperature and Pressure). The BMP test was performed in 500 mL borosilicate glass reactor bottles. The test was performed as triplicate batch experiments for statistical significance and average values were used as an output. The reactor bottles were incubated at the most favorable temperature to methane producing microorganisms i.e. 37°C [10].

Considering the inoculum to substrate ratio as 2.5, each reactor bottle was filled with 5g of VS of inoculum and 2g of VS of crop residue. Afterwards, the reactor bottles were top up to 400 mL volume by adding tap water (filtered surface water). Additional three reactor bottles were filled with inoculum only, to measure the methane production from the inoculum and were considered as blank reactors. In order to control the decrease in pH due to the formation of volatile fatty acids during the AD, supplementary 1.5g of sodium hydrogen carbonate ($NaHCO_3$) was added in each reactor bottle that works as buffer. Then the reactor bottles were hermetically sealed with rubber plugs followed by the plastic screw thread cap to ensure anaerobic condition. The plastic screw thread cap was built in with geared electric motor, which enables to establish the uniform environment throughout the reactor in order to get better interaction between the organic material and microorganisms.

The AD produces biogas, which mainly consists of methane and carbon dioxide gases. In order to measure methane, the carbon dioxide gas was absorbed in three molar solution of sodium hydroxide (NaOH) [11]. Additionally, 3M NaOH also contains 5 mL per liter 0.4% of Thymolphthalein pH indicator. This prepared solution was filled into 100 mL borosilicate glass bottles up to 80 mL. These absorbent bottles were closed hermetically with rubber stoppers and were connected between the reactor bottles and automatic gas measuring device through tygon tubing. Before incubating the reactors bottles, nitrogen gas was purged for 5 minutes to ascertain the anaerobic condition not only in the head space of the reactor bottles but also in the absorbent bottles. The BMP test was stopped after 30 days of incubation as gas generation seized.

2.3 Kinetics of Anaerobic Digestion

The most important variable in kinetics of the AD is the concentration of the substrates with respect to time as the degradation process of the organic material highly depends on it. The general material balance expression for an anaerobic reactor is "Accumulation = Inflow - Outflow + Generation" and symbolically can be written as Equation (1), where S is the concentration of substrate (gVS) at time (t), S0 is the initial concentration of substrate (gVS), Q is the flow of substrate, V is the volume of reactor and R_c is the rate of reaction.

$$\frac{dS}{dt}V = QS_0 - QS + R_cV \tag{1}$$

In present study the kinetics of anaerobically digested crop residues was assessed using batch reactors. As in batch reactor, there is no any inflow or outflow; in other words the flow of substrate is zero i.e. Q=0, thus material balance equation can be modified for batch reactor as Equation (2).

$$\frac{dS}{dt} = R_c \tag{2}$$

First order kinetic models are the simplest models applied to the anaerobic digestion of complex substrates as they provide a simple basis for comparing stable process performance under practical conditions [7]. The literature reveals that the experimental methane production ascertained from the AD can be expressed by a first order reaction kinetics [12-14]. It proceeds at a rate of reaction is directly proportional to the concentration of one of the reactants i.e. R_c= -kxS, which yields Equation (3), where k is the reaction rate constant and generally expressed in per day.

$$\frac{dS}{dt} = -kS \tag{3}$$

Usually, reaction rate constant is determined from the results of batch or continuous flow experiments, by using integration or differential methods. On integration within the limits S_0-S and 0-t, Equation (3) can be rearranged as:

$$S = S_0\, e^{-kt} \tag{4}$$

For AD process, the substrate concentration can be correlated with quantity of methane through the methane yield coefficient (Y_c) as given in Equation (5).

$$Y_c = -\frac{dG}{dS} \tag{5}$$

By integration considering the limits S_0-S and 0-G, Equation (5) can be rearranged as:

$$G = Y_c\,(S_0 - S) \tag{6}$$

Where G is the volume of methane at given time (NmL). Substituting the value of S from Equation (4) into Equation (6), gives Equation (7), where $G_{max} = Y_c\, S_0$ is the maximum volume of methane (NmL) and k is the first order methane generation rate constant (day^{-1}). This equation is an analytical relation between volume of methane generation and AD time and also known as first order exponential model.

$$G(t) = G_{max}\,[1 - e^{-kxt}] \tag{7}$$

The methane production through AD follows four stages i.e. hydrolysis, acidogenesis, acetogenesis and methanogenesis. The methanogenesis is the fourth and final stage of AD, in which methane producing microorganisms (methanogens) transform the acetic acid and hydrogen produced by the acid forming microorganisms to methane and carbon dioxide gases. The methane production also depends upon the growth of methane forming bacteria. If lag phase is considered as in case of bacterial growth curve, or in other words if we in integrate Equation (3) within the limits S_0-S and L-t, then Equation (7) transforms to Equation (8), where L is the lag phase period (day) and denoted the methanogens growth lag phase period. The lag phase period in Equation (8) ranges from zero to few days and is represented as first order exponential model with lag phase period.

$$G(t) = G_{max}\,[1 - e^{-k(t-L)}] \tag{8}$$

Another improvement in first order exponential model can be made by considering that the, rate of the AD process decreases as the methane production increases, which can mathematically expressed as the curve factor in first order exponential model as given in Equation (9), where C is the dimensionless curve factor, appears as the power to the ratio of volume of methane at any time to the maximum volume of methane.

$$\left[\frac{G(t)}{G_{\max}}\right]^{C} = \left(1 - e^{-k\times t}\right) \tag{9}$$

Equation (9) can be rearranged for volume of methane at any time t as Equation (10) and represents the dynamic change of the rate constant happening during the AD process.

$$G(t) = G_{\max} C\sqrt{\left(1 - e^{-k\times t}\right)} \tag{10}$$

In present study for the assessment of the kinetics of the anaerobic digestion, three models i.e. Equations (7-8 and 10) were employed, whereas the values of k, L, C and G_{max} for each model were estimated analytically using a non-linear regression through least square method.

2.4 Comparing Models

The three selected first order models i.e. exponential model, exponential model with lag phase and exponential model with curve factor were compared through three statistical parameters i.e. the coefficient of multiple determinations (R^2), SDR (Standard Deviation of Residuals) and AIC were estimated. These parameters are comprehensive to quantify the accuracy of the mathematical models against the ascertained data. R^2 was calculated by Equation (11); such that $0<R^2<1$, and denotes the strength of the correlation between ascertained methane production (G_{asc}) and modeled methane production (G_{mod}) at time i for n number of days. An equation with a higher R^2 value makes a better estimation.

$$R^2 = 1 - \frac{\sum_{i=1}^{n}\left(G_{asci} - G_{\mathrm{mod}i}\right)^2}{\sum_{i=1}^{n}\left(G_{asci} - \bar{G}_{\mathrm{mod}i}\right)^2} \tag{11}$$

SDR is a measure of the mean difference between values ascertained experimentally and the values estimated by a model and represented in same units of methane production. It was calculated by using Equation (12). The model with the lowest value of SDR makes a better estimation.

$$SDR = \sqrt{\frac{\sum_{i=1}^{n}\left(G_{asci} - G_{\mathrm{mod}i}\right)^2}{n}} \tag{12}$$

AIC is a measure of the relative goodness of fit of statistical models. It balances the change of goodness of model fitting versesthe number of model parameters and was calculated by using Equation (13), where RSS is residual sum of squares, n is number of days, and N is number of model parameters. The model with the lowest value of AIC makes a better estimation.

$$AIC = n\ln\left(\frac{RSS}{n}\right) + 2\left(N+1\right) + \frac{2\left(n+1\right)\left(N+2\right)}{\left(m-N-2\right)} \tag{13}$$

3. RESULTS AND DISCUSSION

3.1 Results of BMP Test

The methane gas production by the ad process not only contingent upon the substrate constitution, but also on the operational parameters, the most important parameters are temperature, pH and volatile fatty acids. These parameters have a direct effect on the microorganisms involved in the anaerobic digestion process. The temperature was kept constant at 37±0.2°C throughout the incubation period. A low pH value within the reactor results an accumulation in VFA (Volatile Fatty Acids), which suppresses anaerobic digestion process, while a high pH value leads to an increase in ammonia nitrogen, which is also toxic to methanogens [15-16], thus decreases the methane production. In AD process, an optimum pH of 6.5-8.5 is required [17] and the threshold of VFA is 4000mg CH_3COOH/L [18]. At the end of 30 days BMP test, the digestate of each reactor bottle was investigated for determination of pH and VFA as per APHA, standard methods [9]. The analysis indicates that the anaerobic batch reactors were within the stable range of pH values ranging from 7.10-7.30, whereas the VFA were observed in the range of 300- 900 mg CH_3COOH/L.

The specific methane production ascertained (G_{asc}) from the crop residues and the inoculum at the incubation of 30 days at the temperature of 37±0.2°C is given in Table 2. The highest specific methane was achieved from wheat straw, i.e. about 172.21 Nml/g VS followed by canola straw, rice straw, cotton stalks, banana plant waste, sugarcane trash and bagasse about 154.49, 128.64, 122.73, 120.60, 119.39 and 116.27 Nml/g VS correspondingly. The specific methane production ascertained from inoculum was 80.09 Nml/g VS.

3.2 Results of Assessment of Kinetics and Comparison of Models

The results of non-linear regression models, statistical analysis of first order exponential model, based on daily BMP test are given in Table 2. The maximum estimated specific methane (G_{max}) was 219.70 Nml/g VS for wheat straw and have standard error of 6.81 Nml/g VS. The subsequent estimated specific methane production was 193.80, 187.40, 151.90, 142.30, 142.30, 141.10, 136.70 and 114.70 Nml/g VS for banana plant waste, canola straw, rice straw, sugarcane trash, cotton stalks, bagasse and inoculum correspondingly. The estimated specific methane productions have almost same fashion as of the ascertained specific methane productions, except of the banana plant waste, which poorly fits through the exponential model. This observation can also be assured from higher values of the standard error and SDR for banana plant waste i.e. 22.66 and 7.35 Nml/g VS respectively. The methane production rate constant was ranging from 0.0720-0.0427 per day for cotton stalks and inoculum respectively. The first order exponential model has R^2 values near to unity but have high values of AIC ranging from 92-128 for bagasse and banana plant waste respectively. The reason for more deviation of ascertained specific methane from the estimated in banana plant waste was due to its higher lag phase period and can be seen from Fig. 2.

The results of non-linear regression, statistical analysis for first order exponential lag phase model, based on daily BMP test results are given in Table 3. The Gmax was ranging from 208.20-98.87 Nml/g VS for wheat straw and inoculum and have lower values of standard error ranging from 2.04-10.30 Nml/g VS, which shows that the exponential lag phase model better estimates the G_{max} than that of the exponential model without considering the lag phase. The exponential lag phase model has also lower values of SDR as compared to the exponential model without lag phase. This finding is also supported by the values of R^2 (Table 3), which is more close to unity. Besides statistical hypothesis testing, the exponential lag phase model is more likely to have generated the data as compared to the simple exponential model as it generates less values of AIC. The lag phase for the crop residue AD was ranging from half day for cotton stalks to one and half day to

TABLE 2. RESULTS OF NON-LINEAR REGRESSION AND STATISTICS ANALYSIS FOR EXPONENTIAL MODEL

Name of Substrate	G_{asc} (NmL/g VS)	G_{max} (NmL/g VS)	SDR (NmL/g VS)	k (1/day)	R^2	AIC
Bagasse	116.27	136.70±4.25	4.06	0.0674±0.0043	0.9884	92
Banana Plant Waste	120.60	193.80±22.66	7.35	0.0364±0.0064	0.9708	128
Canola Straw	154.49	187.40±5.63	4.63	0.0615±0.0036	0.9913	100
Cotton Stalks	122.73	141.10±2.70	2.87	0.0720±0.0029	0.9945	70
Rice Straw	128.64	151.90±4.50	4.55	0.0698±0.0043	0.9883	99
Sugarcane Trash	119.39	142.30±3.74	3.21	0.0632±0.0032	0.9929	77
Wheat Straw	172.21	219.70±6.81	4.51	0.0542±0.0030	0.9934	98
Inoculum	80.09	114.70±9.67	4.21	0.0427±0.0058	0.9765	94

TABLE 3. RESULTS OF NON-LINEAR REGRESSION AND STATISTICS ANALYSIS FOR EXPONENTIAL LAG PHASE MODEL

Name of Substrate	G_{max} (NmL/g VS)	SDR (NmL/g VS)	k (1/day)	L (day)	R^2	AIC
Bagasse	129.90±2.91	3.16	0.0798±0.0045	0.789±0.157	0.9932	78
Banana Plant Waste	160.20±10.30	5.70	0.0547±0.0066	1.547±0.281	0.9830	114
Canola Straw	179.20±4.55	3.98	0.0704±0.0042	0.611±0.168	0.9938	92
Cotton Stalks	136.50±2.04	2.25	0.0807±0.0031	0.532±0.112	0.9967	57
Rice Straw	145.10±3.23	3.64	0.0817±0.0046	0.745±0.161	0.9928	87
Sugarcane Trash	135.50±2.60	2.45	0.0734±0.0034	0.672±0.130	0.9960	62
Wheat Straw	208.20±5.30	3.75	0.0625±0.0034	0.614±0.151	0.9956	88
Inoculum	98.87±4.70	3.14	0.0606±0.0058	1.393±0.234	0.9873	77

banana plant waste. The accuracy of the exponential lag phase model over simple exponential model can also be depicted through Figs. 1-8, as the estimated methane through exponential lag phase model are more closer to the ascertained methane. Because of the addition of the lag phase, Equation (8) estimates higher values of the methane production rate constant and was ranging from 0.0547-0.0817 per day.

The results of non-linear regression, statistical analysis for first order exponential curve factor model, based on daily BMP test results are given in Table 4. The G_{max} was ranging from 186.60-80.91 Nml/g VS for wheat straw and inoculum and have even lower values of standard error ranging from 0.69-2.28 Nml/g VS than that of the exponential lag phase model, which shows that the exponential curve factor model not only better estimates the G_{max} than that of the simple exponential model but also to the exponential lag phase model. The accuracy of the exponential curve factor model over simple exponential model and exponential lag phase model can also be observed through Figs. 1-8, as the estimated methane through exponential curve factor model almost follows the experimental methane line. Moreover, the SDR is also low for exponential curve factor model and was ranging from 0.98-2.08 Nml/g VS. The curve factor for the crop residue AD was ranging from 0.3549-0.7084. Besides statistical hypothesis testing, the exponential curve factor model is more likely to have generated the data as compared to the other models under present study as it generates lesser values of AIC. This also establishes that the BMP test is convenient in the kinetic studies of the crop residues digested with buffalo dung. Because of the involvement of the curve factor, Equation (10) estimates higher values of the methane production rate constant even than that of the Equation (8) and was ranging from 0.096-0.143 per day.

TABLE 4. RESULTS OF NON-LINEAR REGRESSION AND STATISTICS ANALYSIS FOR EXPONENTIAL CURVE FACTOR MODEL

Name of Substrate	G_{max} (NmL/g VS)	SDR (NmL/g VS)	k (1/day)	C	R^2	AIC
Bagasse	118.50±0.87	1.27	0.1290±0.0042	0.5808±0.0201	0.9989	21
Banana Plant Waste	124.90±0.89	1.27	0.1433±0.0039	0.3549±0.0132	0.9992	21
Canola Straw	162.00±1.83	2.08	0.1110±0.0049	0.6410±0.0268	0.9983	52
Cotton Stalks	128.00±0.93	1.13	0.1118±0.0034	0.7084±0.0197	0.9992	14
Rice Straw	133.70±1.32	1.90	0.1271±0.0055	0.6026±0.0274	0.9980	46
Sugarcane Trash	124.70±1.20	1.32	0.1081±0.0041	0.6718±0.0230	0.9988	24
Wheat Straw	186.60±2.28	2.00	0.0966±0.0042	0.6796±0.0244	0.9987	49
Inoculum	80.91±0.69	0.98	0.1381±0.0047	0.4098±0.0175	0.9988	5

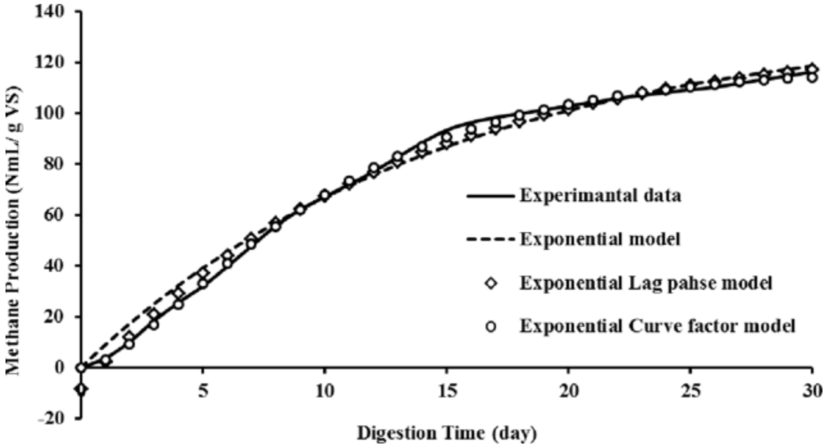

FIG. 1. EXPERIMENTAL AND ESTIMATED METHANE PRODUCTION FOR BAGASSE

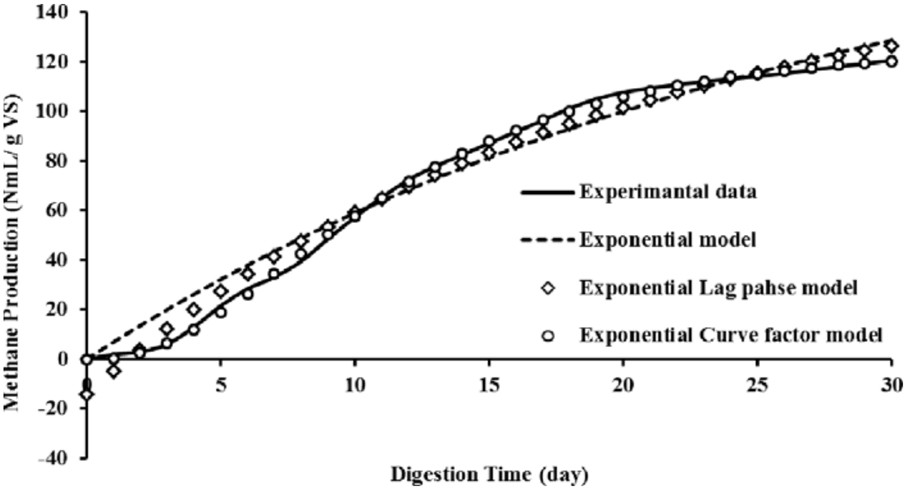

FIG. 2. EXPERIMENTAL AND ESTIMATED METHANE PRODUCTION FOR BANANA PLANT WASTE

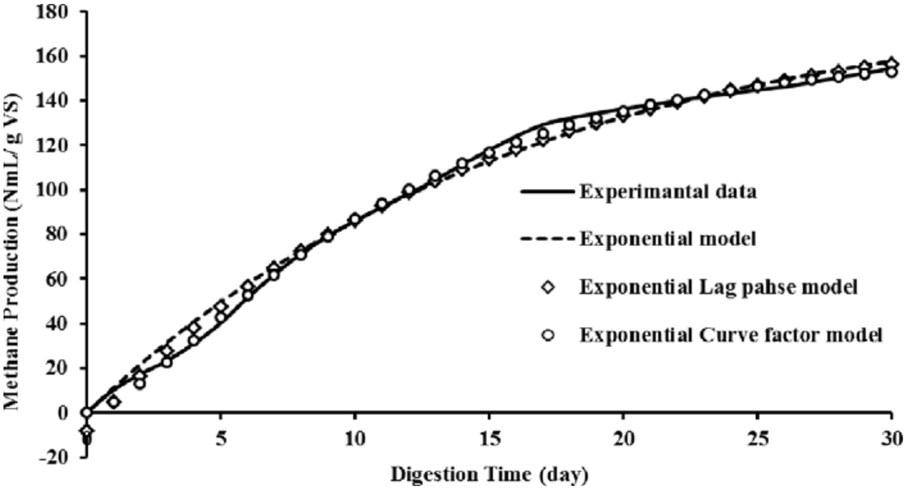

FIG. 3. EXPERIMENTAL AND ESTIMATED METHANE PRODUCTION FOR CANOLA STRAW

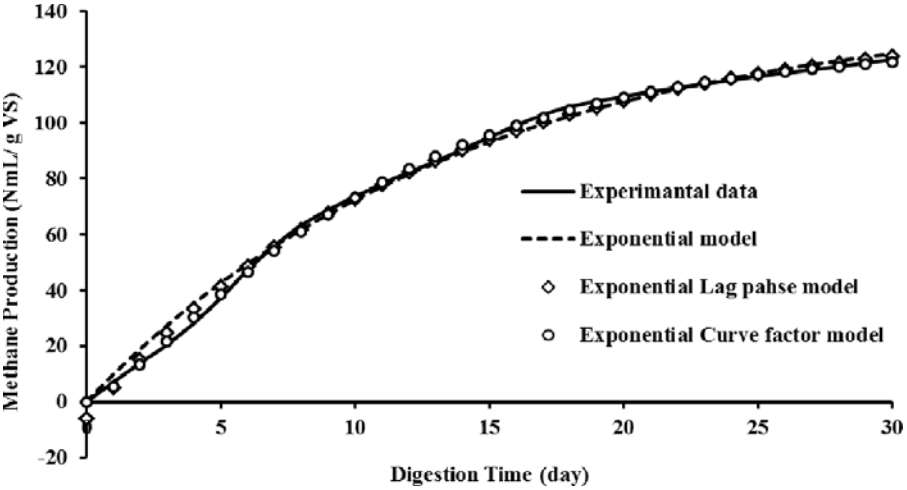

FIG. 4. EXPERIMENTAL AND ESTIMATED CUMULATIVE CH4 PRODUCTION ALONG WITH FLOW RATE FOR COTTON STALKS

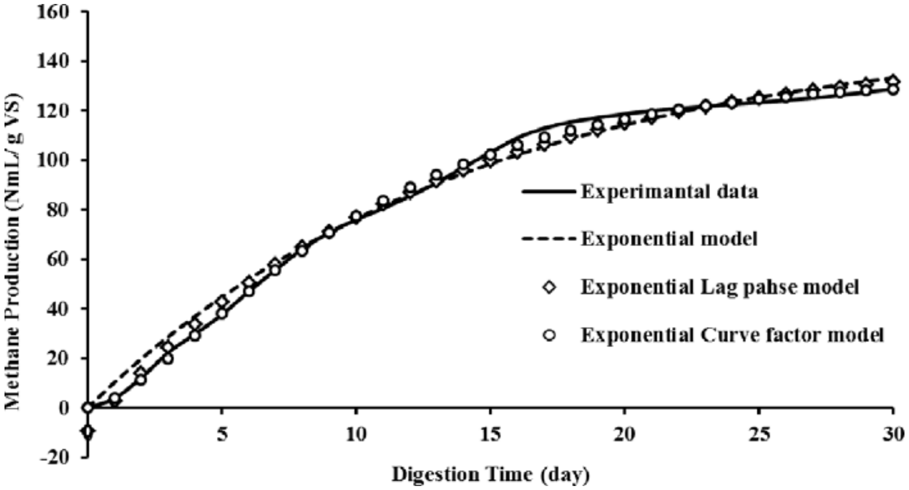

FIG. 5. EXPERIMENTAL AND ESTIMATED METHANE PRODUCTION FOR RICE STRAW

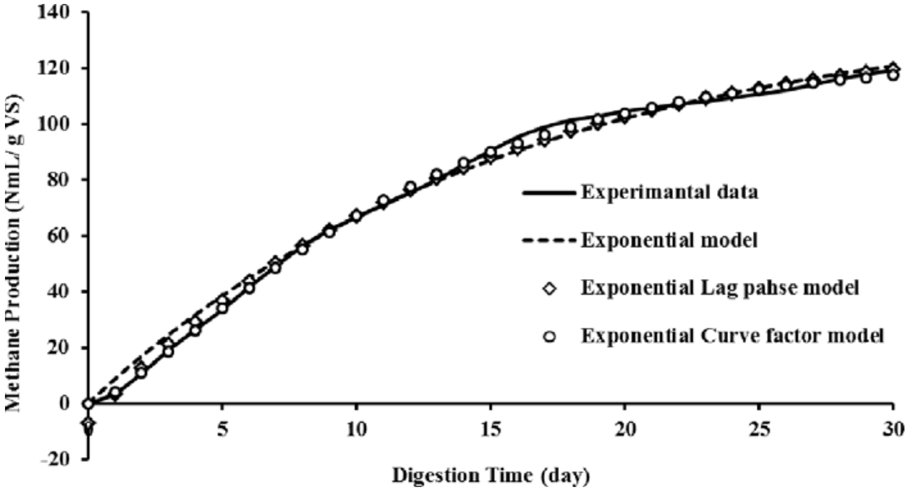

FIG. 6. EXPERIMENTAL AND ESTIMATED METHANE PRODUCTION FOR SUGARCANE TRASH

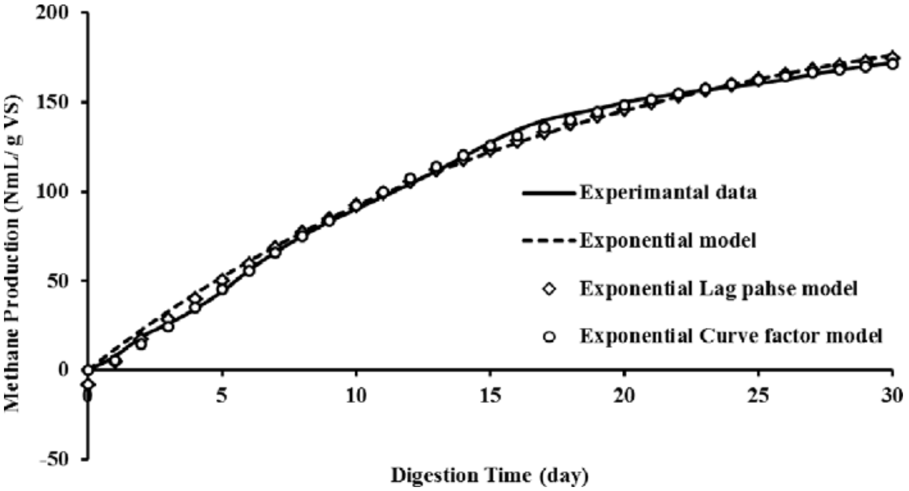

FIG. 7. EXPERIMENTAL AND ESTIMATED METHANE PRODUCTION FOR WHEAT STRAW

Out of the three models, the simple exponential model was the poorest model to estimate the methane production and do not obey Equation (7), which is in agreement with Sworakowski and Matczyszyn [19]. The first order exponential curve factor model gives better accuracy. The curve factor is more significant to the lag phase, because the reactor bottles in the BMP test were not only kept at favorable temperatures of the methanogens but also added methanogens culture, which was established at the same environment. Moreover, the purging of nitrogen before starting the BMP test removes molecular oxygen, thus the methane production was started from day one and decreases the lag phase as can be observed from Table 3 and from Figs. 1-8.

The disadvantage of exponential lag phase model is the non-zero behavior at time t=0, whereas in real circumstances when t=0, not only the cumulative methane production is zero but also the biogas. On the contrary, the exponential curve factor model generates zero at time t=0. Additionally, the advantage of the curve factor includes wiggling effect in the estimated curve or in other words it increases the inflection point, which may cause reduction of sum of squares, thus fits well than the other models. If the exponential model will be included both the lag phase period and curve factor, even then the model will have non-zero behavior, which not only deviate the estimated curve from the data points ascertained, but also generates higher AIC differences, thus poorly fits the model.

The correlation between the lag phase period and the curve factor is illustrated in Fig. 9. It shows that there is an inverse linear relationship between the lag phase period and the curve factor as the R^2 is near to unity.

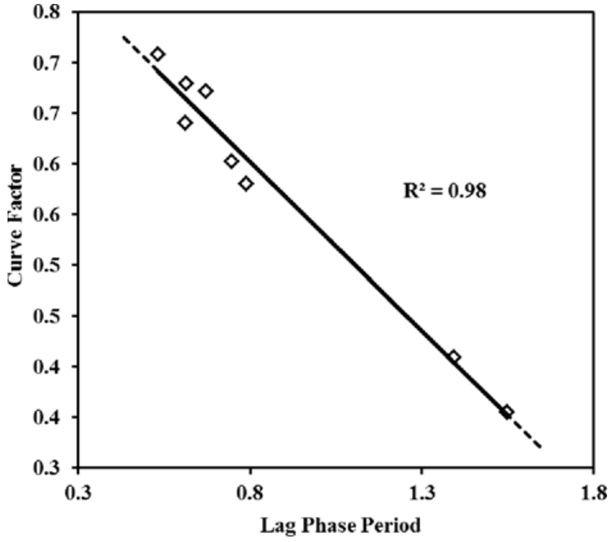

FIG. 9. CORRELATION BETWEEN LAG PHASE PERIOD AND CURVE FACTOR

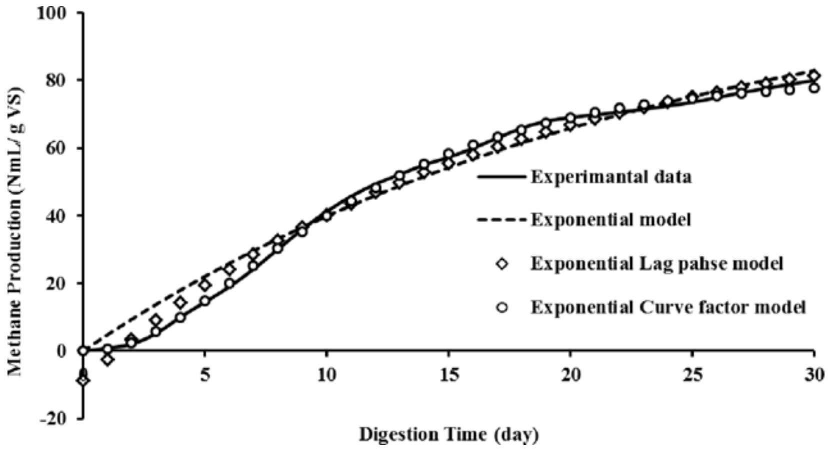

FIG. 8. EXPERIMENTAL AND ESTIMATED METHANE PRODUCTION FOR INOCULUM

4. CONCLUSIONS

The present study reveals that, the AD of crop residues and buffalo dung follows the first order kinetics. The experimental specific methane productions from all seven selected crop residues were in better agreement with estimated specific methane productions, thus establishes that the BMP test is convenient method to study the kinetic. Out of the three first order exponential models, the simple exponential model was the poorest model to estimate the methane production. In comparison to the lag phase and curve factor, the later gives better accuracy if included in the first order exponential model. In addition to statistical hypothesis testing, the exponential curve factor model is more likely to have generated the data as compared to the other models under present study as it has least AIC values. Present study findings also includes that there is an inverse linear relationship between the lag phase period and the curve factor.

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